Hepatitis B virus X protein: a multifunctional viral regulator

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Hepatitis B Virus (HBV) infection is one of the major causes of hepatocellular carcinoma (HCC). X protein (HBx) has been suspected to be oncogenic, although the precise role(s) remain uncertain. HBx is a multifunctional viral regulator that modulates transcription, cell responses to genotoxic stress, protein degradation, and signaling pathways. These modulations affect viral replication and viral proliferation, directly or indirectly. HBx also affects cell cycle checkpoints, cell death, and carcinogenesis. This article presents an overview of the progress in HBx research over the past several years.

Key words: HBV, X protein (HBx), hepatocellular carcinoma, transactivation

Hepatitis B virus X protein: promiscuous or multifunctional

Hepatitis B Virus (HBV) X protein (HBx) has been the focus of much attention in recent years, because it is regarded as strongly implicated in hepatocarcinogenesis. X gene encodes 154 amino acids that have no counterparts in hosts, and is conserved among mammalian hepadnaviruses. X protein has been shown to be essential for viral proliferation in the woodchuck although its essential function in viral proliferation remains to be explored. HBx augments HBV pregenome transcription and HBV replication. Modulatory functions of HBx have been documented in terms of transcription, signaling pathways, genotoxic stress responses, protein degradation, cell-cycle control and carcinogenesis. Target processes and factors with which HBx is involved are still accumulating. The cynical statement that “if there is a protein that is not among the list of HBx interactors, then this protein has probably not yet been tested” reflects one aspect of the field. However, the controversial and diverse outcomes in the field partly reflect the properties of HBx itself, because HBx, in particular the transactivation domain, has the capacity to interact with many proteins both in vivo and in vitro. The findings thus far may partly reflect scientists’ behavior; “To find a novel thing is better than to deepen the finding”. Nevertheless, knowledge of HBx has been accumulating by continuous efforts in the field, and HBx is regarded as a multifunctional protein that is important for the viral life cycle and viral-host interactions. This article reviews the recent progress in HBx research in the several years since the previous review.

Expression of HBx

The expression of HBx is regulated primarily at a transcriptional level, under the control of Enhancer I (Enh I), which harbors more than a dozen cis-elements for the ubiquitous and tissue-specific transcriptional factors within approximately 400 bp. HBx transactivates Enh I through AP1 and C-stretch elements within the Enh I core sequence. Enh I regulates all the HBV promoters (X-, PreS/S-, and PreC-C/pregenome-promoters) and Enhancer II regulates the PreC-C/pregenome promoter. X expression is under autoregulation, and is also regulated by a variety of signaling pathways in inflammation and tumor-promoting responses. Enh I has been further documented as the target of several signaling pathways. The upregulation of Enh I was reported to occur by insulin through AP1 in the Enh I core, and by interleukin (IL)6 through the AP-1 and C-stretch elements to which overexpressed nuclear factor (NF)-IL6 can bind. Lee et al. observed that HBx stable transfected cells induced angiogenesis under hypoxic conditions, in which the vascular endothelial growth factor (VEGF) promoter was upregulated, together
with the augmented expression of HBx through the AP-1 and C-stretch in the Enh I core. Their finding opens the possibility that HBx may play a critical role in hypoxia-induced angiogenesis during hepatocarcinogenesis. The downregulation of Enh I by a genotoxic response through a novel p53-binding site in the vicinity of Enh I has been reported.\textsuperscript{11,12} The pregenome/core promoter is responsive to HBx in transgenic mice,\textsuperscript{13} and a similar transactivation of HBx was reported in cultured cells to augment the Enh II/pregenome promoter through a C/EBP binding site in the Enh II/Pregenome promoter.\textsuperscript{14} However, the recruitment of hormone receptor proteins (HNF4, RXR, and PPAR) to the Enh II/pregenome promoter has recently been reported in the regulation of HBV replication in nonhepatic and hepatic cells.\textsuperscript{15}

### Subcellular localization of HBx

The subcellular localization of HBx has been controversial, but the general consensus is that most HBx is in the cytoplasm, with a small fraction in the nucleus, especially when HBx is ecotropically expressed in cells. In naturally infected hepatocytes, Dandri et al.\textsuperscript{16} clearly demonstrated the cytoplasmic and nuclear matrix localization of WHV X protein with different half-lives. A similar bimodal turnover of HBx was observed in human cultured cells.\textsuperscript{17} The capacity of HBx for nuclear localization may be limited, and most overexpressed HBx was found exclusively in two distinct populations in cytoplasm; a nonmitochondrial population and a mitochondrial one.\textsuperscript{18}

Two groups have reported that the mitochondria is another target for HBx.\textsuperscript{19,20} Takada et al.\textsuperscript{19} claimed abnormal aggregations of mitochondria and several types of mitochondrial dysfunction related to cell death. Ramahni et al.\textsuperscript{20} reported that a mitochondrial protein, voltage-dependent anion channel (HVDAC3), directly interacted with HBx, and this affected mitochondrial transmembrane potential. However, these phenomena may occur when HBx is overexpressed, as reported by Henkler et al.\textsuperscript{18}

HBx has been reported to interact with a variety of proteins: cytoplasmic, nuclear, and those that traffic between the cytoplasm and the nucleus, as shown in Fig. 1. Artificial taggings of nuclear localization signal (NLS) to HBx were found to result in nuclear localization of HBx in some reports,\textsuperscript{21,22} but we observed only a weak tagging effect on the subcellular localization of HBx.\textsuperscript{23} Recently, Forgues et al.\textsuperscript{24} addressed the effect of the nuclear export pathway on the subcellular localization of HBx. A functional NES (nuclear export signal) was found in the C-terminal part of HBx, and substitution mutations (L98A and L100A) resulted in the nuclear distribution of HBx. Furthermore, they demonstrated that HBx activated nuclear factor kappa B (NF-xB) by inducing its nuclear translocation in an NES-dependent manner.\textsuperscript{24} These results suggest that the nuclear localization of HBx may be under the control of the nuclear export pathway. In this context, Weil et al.\textsuperscript{25} demonstrated that the direct interaction of HBx and I kappa B alpha (IkBa) resulted in the nuclear distribution of the two proteins and NF-xB activation. Similarly, the interaction between HBx and Smad4 was reported to affect the subcellular trafficking of Smad4, thus enhancing transforming growth factor (TGF)-\beta signaling.\textsuperscript{26}

These reports clearly suggest that the interaction between HBx and trafficking proteins may modulate their functions by modulating the nuclear delivery of the fac-

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**Fig. 1.** Direct targets of hepatitis B virus X protein (HBx). The reported targets of HBx are shown schematically. Proteins that are reported to interact directly with HBx are shown in boldface. The factors that traffic between the cytoplasm and nucleus are shown in the small box (labelled shuttling factors). See text for reports on these factors.